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Limitations of Fibrinogen-Polymyxin Medium in Detecting Coagulase-Positive Staphylococci in Raw Milk[1]

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ABSTRACT

McDIVITT, MAXINE E. (University of Wisconsin, Madison), AND NORGE W. JEROME. Limitations of fibrinogen-polymyxin medium in detecting coagulase-positive staphylococci in raw milk. Appl. Microbiol. **13**:157-159. 1965.—A fibrinogen-polymyxin medium and Staphylococcus Medium 110 were used in the isolation of coagulase-positive staphylococci in raw milk. Results indicated that both media allow the growth of some rods and of many coagulase-negative cocci. A significantly greater number of coagulase-positive staphylococci were identified by the tube test than were revealed by halo formation on fibrinogen-polymyxin medium.

The development and evaluation of media for use in the rapid detection of coagulase-positive staphylococci has received the attention of many workers. Several approaches stimulated by this problem were recently compared by McDivitt and Topp (1964).

Fibrinogen or plasma on the surface of selective media was shown by Deneke and Blobel (1962) to be useful in the presumptive identification of coagulase-positive staphylococci. Coagulase elaborated by the organisms converts fibrinogen to fibrin and forms halos or opaque zones around colonies. These workers reported a close correspondence between the characteristic halo reactions on two fibrinogen agar media and coagulase tube tests when a number of pure cultures of staphylococci were examined. Recovery of *Staphylococcus aureus* was not quantitative in mixed cultures despite the selectivity of the media for the organisms.

The study reported here was concerned with the ability of a fibrinogen medium to indicate the presence of coagulase-positive staphylococci in raw milk. A second purpose was to compare results with those obtained by simultaneous plating on Staphylococcus Medium 110 (SM 110), a medium widely used for isolation of coagulase-positive staphylococci.

MATERIALS AND METHODS

The base for the fibrinogen-polymyxin medium (FPM) contained the following ingredients (per liter): 25.0 g of Heart Infusion Broth (Difco), 17.5 g of agar (Difco), 0.4 g of cycloheximide (The Upjohn Co., Kalamazoo, Mich.), and 10.0 mg of polymyxin B sulfate (Burroughs-Wellcome and Co., Tuckahoe, N.Y.). A 0.5-ml portion of fibrinogen preparation was added per plate. The fibrinogen preparation was made by sterilizing 3% bovine fibrinogen (Armour Pharmaceutical Co., Kankakee, Ill.) in physiological saline by Seitz filtration; 3% (by volume) of sterile rabbit plasma (Colorado Serum Co., Denver, Colo.) was added after filtration.

Samples of mixed raw milk were obtained over a period of 3 months from the processing plant of the Department of Dairy and Food Industries at the University of Wisconsin. Samples were diluted with a 0.1% peptone dispersion (Difco) and plated in duplicate on FPM and on SM110. Plates were incubated at 37 C for 48 hr. Plate Count Agar (PCA) was used in the determination of total numbers of bacteria present.

Morphology of organisms isolated. The ratio of rods to cocci in raw milk was determined by the examination of stained smears of all colonies on randomly selected portions of PCA plates. Smears of all colonies removed from the selective media were also prepared and stained. Examination revealed the ability of FPM and SM110 to select cocci from a mixed culture.

Presumptive evidence of the presence of coagulase-positive staphylococci on FPM. FPM plates were examined for halo formation 24 hr after incubation and replaced in the incubator to permit full colony development for other investigations. Preliminary studies had shown that, under the conditions of this study, incubation for 24 hr was optimum for determining the number of halos formed.

Confirmatory tests for the presence of coagulase-

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TABLE 1. *Selective action of fibrinogen-polymyxin medium and Staphylococcus Medium 110 for coagulase-positive staphylococci in raw milk**

Sample	Cocci on Plate Count Agar	Fibrinogen-polymyxin medium		Staphylococcus Medium 110	
		Cocci	Coagulase-positive cocci (tube test)	Cocci	Coagulase-positive cocci (tube test)
	%	%	%	%	%
7	33	67	17	87	44
8	28	91	36	100	6
9	72	95	35	73	4
10	39	86	38	100	40
11	14	71	29	92	17
12	16	96	21	100	22
13	18	43	14	88	0

* Percentages are based on averages of colonies picked from randomly selected portions of duplicate plates.

positive staphylococci on FPM and SM110. All visible, removable colonies were picked from randomly selected one-half portions of FPM and SM110 plates containing samples of comparable sample dilution. These were inoculated into Brain Heart Infusion (Difco) and incubated at 37 C for 18 hr. After incubation, tube coagulase tests were performed by adding 0.2 ml of the broth culture to 0.5 ml of fibrinogen-plasma preparation [1.5% (w/v) bovine fibrinogen and 1.5% rabbit plasma in physiological saline]. At the time of transfer, the colonies which had shown halo development were identified.

Analysis of data. Three analyses of variance, as described by Steele and Torrie (1960), were made on the data obtained in this study. The selective response of the agar media in isolating cocci from mixed cultures and in indicating coagulase activity of the organisms isolated was analyzed. Another analysis compared the percentage of colonies which developed halos on FPM with the percentage which gave a positive reaction by the tube coagulase test.

RESULTS AND DISCUSSION

Table 1 indicates the percentage of cocci which appeared on the three media. All percentages are based on averages of the number of colonies picked from portions of duplicate plates. It can be assumed that the findings on PCA reflect the extent to which cocci occurred in the raw milk and the level of competing organisms in the flora. The difference in the selective action of FPM and SM110 for coagulase-positive cocci in raw milk was not statistically significant. Both media allowed the growth of some rods and of many cocci which were not coagulase-positive.

TABLE 2. *Comparison of two methods in detecting the presence of coagulase-positive staphylococci in raw milk*

Sample	Medium*	No. of colonies tested	Tube test for coagulase activity		Halo formation as indication of coagulase activity	
			No. positive	Per cent positive	No. present	Per cent present
1	FPM	13	2	15.4	1	7.7
	SM110	21	4	19.0		
2	FPM	20	8	40.0	2	10.0
	SM110	18	2	11.1		
3	FPM	37	15	40.5	4	10.8
	SM110	30	8	26.6		
4	FPM	29	9	31.1	3	10.3
	SM110	39	9	23.1		
5	FPM	16	6	37.5	1	6.2
	SM110	29	10	34.5		
6	FPM	19	5	26.3	3	15.8
	SM110	38	9	23.7		
7	FPM	18	3	16.6	0	0.0
	SM110	9	4	44.4		
8	FPM	22	8	36.4	2	9.1
	SM110	17	1	5.9		
9	FPM	20	7	35.0	1	5.0
	SM110	23	1	4.3		
10	FPM	13	5	38.4	4	30.8
	SM110	15	9	40.0		
11	FPM	24	7	29.2	3	12.5
	SM110	12	2	16.6		
12	FPM	47	10	21.1	3	6.4
	SM110	18	4	22.2		
13	FPM	21	3	14.3	1	4.8
	SM110	8	0	0.0		

* FPM = fibrinogen-polymyxin medium; SM110 = Staphylococcus Medium 110.

Table 2 indicates in more detail the ability of FPM and SM110 to isolate coagulase-positive staphylococci in raw milk; it also shows the lack of efficiency of the halo reaction on FPM in quantitatively detecting the presence of these organisms. A greater percentage of the colonies which grew on FPM produced coagulase than was indicated by halo formation. This difference was significant at the 1% level and indicates that when the medium is used with raw milk the halo count underestimates the number of coagulase-positive staphylococci present. The absence of halos can, therefore, not be regarded as an indication of the absence of enterotoxigenic staphylococci. These findings agree with those reported by Deneke and Blobel (1962) for contrived mixed flora.

The medium used by Deneke and Blobel (1962) contained 75 mg of polymyxin per liter. McDivitt and Topp (1964) reported that medium containing this quantity of polymyxin was inhibitory to pure cultures of *S. aureus* cultivated in sterile milk; the inhibitory effect was not found with the lower concentration of polymyxin used in the present study. This suggests that the inhibition of the development of halos recorded in Table 2 cannot be attributed to the level of polymyxin used.

Fresh rabbit plasma was used by Deneke and Blobel (1962). To ascertain whether the low recovery of coagulase-positive staphylococci could be attributed to the use of the commercially prepared product, control plates made with fresh plasma were included in a number of trials. The comparable counts obtained indicated that the source of plasma did not influence the results.

The predominance of rods and the presence of coagulase-negative cocci may act to suppress halo development on FPM. Deneke and Blobel's (1962) report permits speculation on the interaction of both quantitative and qualitative effects of competing organisms on *S. aureus*. Further study is necessary to clarify the role of interfering agents or conditions on the halo reaction as a means of indicating the presence of enterotoxigenic staphylococci in raw milk. The small quantity of sample used with this medium and the low recovery of organisms giving the characteristic reaction limit its usefulness.

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